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Aggregation of Azo Dyes with Cationic Amphiphiles at Low Concentrations in Aqueous Solution

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The aggregation of a series of *n*-alkyltrimethylammonium bromide (C_n TAB, $n = 10, 12, 14, 16$, and 18) and 4-*n*-alkyl-1-methylpyridinium iodide amphiphiles (C_m pyI, $m = 8, 10, 12$, and 14) induced by low concentrations of azo dyes in aqueous solution has been investigated by means of ultraviolet (UV) spectroscopy. It was observed that aggregation takes place at surfactant concentrations far below the cmc of C_{12} TAB, C_{14} TAB, C_{16} TAB, and C_{18} TAB with methyl orange (MO), ethyl orange (EO), and *para*-methyl red (pMR). Aggregation below the cmc of C_{10} TAB was also induced by EO. In the case of MO and pMR, however, higher dye concentrations were necessary to induce aggregation. Interactions at low surfactant concentrations have also been observed in aqueous solutions of C_{10} pyI, C_{12} pyI, and C_{14} pyI with MO. Binding of methyl red (MR), methyl yellow (MY), and azobenzene sulfonate (ABS) with cationic surfactants below their cmc did not occur. Aggregation was reflected by the appearance of a blue-shifted absorption band in the spectra of the dyes. Precipitates formed in aqueous solutions from the cationic surfactants and MO and pMR are C_n TA–MO, C_n TA–pMR, and C_m py–MO salts and consist of an equimolar ratio of surfactant and dye. In similar experiments with EO, MR, MY, or ABS as the solvatochromic dye molecules, no precipitation occurred, although surfactant–EO salts precipitated at high EO concentration. Dye–surfactant salts formed from C_n TAB and MO were found to form myelins in phase penetration experiments. Temperatures at which myelins start to form increase upon increasing alkyl chain length of the surfactant as revealed by optical microscopy. The formation of vesicles from C_{10} TA–MO and from C_{12} TA–MO crystals was indicated by electron microscopy. The presence and position of the ionic group in the dye molecule is important in determining the association. The importance of hydrophobic interactions is revealed by the chain length dependence on the aggregation process and the observation that interactions are absent in ethanol. Electrostatic interactions also play an important role, as shown by the effect of NaCl on the binding process.

Introduction

The binding of dye molecules to proteins has been extensively studied by spectral techniques. The data provide insight into small molecule–macromolecule interactions, which are of major importance in biochemistry.¹ Particularly the interactions between cationic dye molecules and anionic proteins² as well as those of anionic dyes with cationic proteins³ have been the subject of numerous studies. Changes in the absorption or fluorescence spectrum of the dye upon binding have been attributed mainly to electrostatic interactions.⁴ Marked changes also occur in the spectra of organic anions when synthetic polymers are added. Charged^{5–7} as well as nonionic^{8–10} polymers have been shown to interact with azo dyes such as methyl orange (MO). It has been shown

that interactions between cationic polymers and MO induce marked reductions in reduced viscosity.^{11,12} Not only polymers are able to induce spectral changes but also surfactants have been shown to aggregate with dye molecules in aqueous solution. Cationic surfactants induce changes in the spectra of anionic dyes such as 2-naphtholate,¹³ bromophenol blue,¹⁴ dansylglycine,¹⁵ and MO.^{11,16} Also, anionic surfactants interact with cationic dyes.¹⁷ Interestingly, the largest spectral changes are observed at surfactant concentrations far below the cmc. The origin of the spectral shifts is still a matter of debate in the literature. Dye aggregation was held responsible for the occurrence of the new band in the case of MO in aqueous solutions of cationic polymers.^{6,7} Upon increasing polymer concentration, dilution of the dye bound to the polymer occurs and the MO spectrum shifts from that of aggregates

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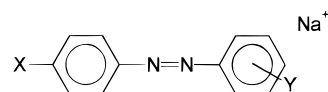
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to that of monomers. Different polymers, however, give rise to different shifts in the wavelength of maximum absorption of MO. It was therefore postulated that the larger shift was caused by the formation of higher-order dye aggregates. The formation of MO dimers has erroneously been excluded on the basis of circular dichroism experiments.¹⁸ Another interpretation was provided by Quadrioglio et al.¹⁶ who postulated a conformational change from the trans to the cis form of the dye molecule in order to explain the appearance of the short-wavelength absorption band. However, using resonance Raman spectroscopy it has been shown that the dye molecule retains its trans form upon binding to proteins and cationic surfactants.¹⁹ More recently, Dutta and Bhat²⁰ proposed the formation of "water-structure-enforced ion pairs in which the headgroup of the surfactant molecule is attached to the sulfonate group of MO" in order to explain the large blue shift in the spectrum of MO upon addition of small amounts of a cationic amphiphile. A short-wavelength absorption band has also been observed upon addition of inorganic salts (e.g., CaCl_2) to MO,^{18,21} but simultaneously, a precipitate is formed, which was thought to be the origin of the blue-shifted absorption band.

In addition to proteins and polyelectrolytes, dyes have also been employed as probes for the micropolarity of cyclodextrines,²² reverse micelles,²³ microemulsions,²⁴ liquid crystals,²⁵ bilayers,²⁶ and monolayers.²⁷ The short-wavelength absorption band of MO has been reported in studies of monolayers²⁷ and microemulsions²⁴ where MO was used as a solvatochromic reporter molecule. In the latter case, the appearance of the short-wavelength absorption band was attributed to dye aggregation. In another study, the incorporation of alcohols into cationic micelles has been investigated using MO as a reporter molecule.²⁸

In the present study, we have investigated the interactions of a series of *n*-alkyltrimethylammonium bromide and 4-*n*-alkyl-1-methylpyridinium iodide amphiphiles with methyl orange, ethyl orange, methyl red, *para*-methyl red, methyl yellow, and azobenzene sulfonate (Chart 1) in aqueous solution. Aggregation was monitored by UV-vis spectroscopy, and the effect of alkyl chain length on the aggregation process was examined. Also, effects of structural variations of the azo dyes on the aggregation process have been investigated. Dye-surfactant salts were studied by optical microscopy, conductometry, and electron microscopy with the aim of characterizing the morphology of the aggregates formed in aqueous solution.

Chart 1. Structures of the Azo Dyes

dye	X	Y
methyl orange (MO)	$(\text{CH}_3)_2\text{N}$	p-SO_3^-
ethyl orange (EO)	$(\text{C}_2\text{H}_5)_2\text{N}$	p-SO_3^-
<i>para</i> methyl red (pMR)	$(\text{CH}_3)_2\text{N}$	p-CO_2^-
methyl red (MR)	$(\text{CH}_3)_2\text{N}$	o-CO_2^-
methyl yellow (MY)	$(\text{CH}_3)_2\text{N}$	H^a
azobenzene sulfonate (ABS)	H	p-SO_3^-

^aNo sodium cation.

Experimental Section

Materials. C_{10}TAB was purchased from Lancaster, C_{12}TAB from Sigma Chemical Co., C_{14}TAB and dodecylamine from Aldrich, C_{16}TAB from Merck, and C_{18}TAB from Fluka. Dodecylamine hydrochloride (DAHCl) was prepared by dissolving dodecylamine in water and adding 1 equiv of HCl. The pH of DAHCl solutions was adjusted to 6. The purity of all surfactants was checked by ^1H NMR. C_{10}TAB and C_{12}TAB were crystallized from acetone. All surfactants, except C_{18}TAB , were dried in vacuo before use. C_8pyI , C_{10}pyI , C_{12}pyI , and C_{14}pyI were synthesized according to a literature procedure.²⁹

Methyl orange (MO), ethyl orange (EO), methyl yellow (MY), and *para*-methyl red (pMR) were obtained from Acros Organics. Methyl red (MR) and disodium tetraborate decahydrate were purchased from Merck. Azobenzene was obtained from Aldrich. MO was crystallized from doubly distilled water. EO was dried in vacuo during one night. Azobenzene sulfonate (ABS) was synthesized according to a literature procedure.³⁰ Water was demineralized and distilled twice in an all-quartz distillation unit.

^1H NMR spectra were measured at 200 or 300 MHz on a Varian Gemini-200 or a Varian VXR-300 spectrophotometer, respectively.

UV-vis Spectroscopy. UV-vis absorption spectra were recorded on a Philips PU 8740 UV-vis spectrophotometer, a Perkin-Elmer $\lambda 5$, or a Perkin-Elmer $\lambda 12$ spectrophotometer, equipped with a thermostated cell compartment. MO, MR, pMR, and ABS concentrations were $25\ \mu\text{M}$. The EO concentration was $23\ \mu\text{M}$, and that of MY was $12\ \mu\text{M}$. All solutions were prepared in 0.02 M sodium borate buffers adjusted to pH 9.4.

Optical Microscopy. Melting points were determined on a Kofler hot stage or a Mettler FP 2 melting point apparatus equipped with a Mettler FP 21 microscope. Phase penetration experiments were performed on an Olympus BX 60 polarization microscope equipped with a Linkam THMS 600 hot stage.

Conductivity Experiments. Critical aggregation concentrations were determined by conductivity experiments. Conductivities were determined on a Wayne-Kerr Autobalance Bridge B642 fitted with a Philips electrode PW 9512101 with a cell constant of $0.71\ \text{cm}^{-1}$. Solutions in the conductivity cell were stirred magnetically and thermostated at the desired temperature. Concentrations were corrected for volume changes.

Electron Microscopy. Transmission electron micrographs were obtained using a JEM 1200 EX electron microscope operating at 80 kV. Samples were prepared on carbon-coated collodion grids. Samples were stained with 1% uranyl acetate.

$\text{C}_{16}\text{TA-MO}$. The precipitate formed from a solution of C_{16}TAB and MO was analyzed. Anal. Calcd for the 1:1 adduct,

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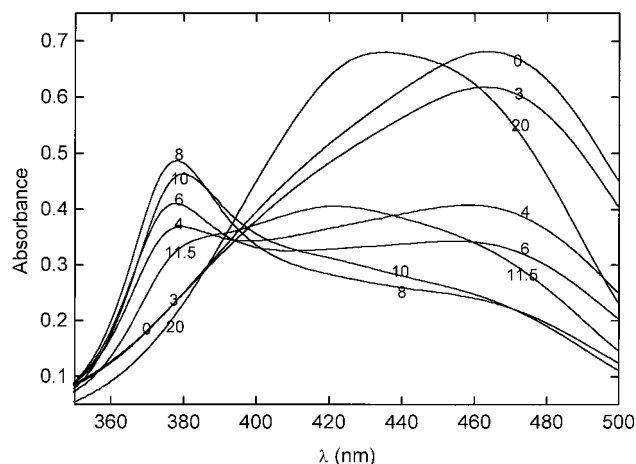


Figure 1. Effect of C_{12} TAB on the absorption spectrum of MO in aqueous solution at 30 °C, pH 9.4, $[MO] = 25 \mu M$. Numbers correspond to surfactant concentrations (mM).

$C_{33}H_{56}N_4SO_3$ (588.89): C, 67.31; H, 9.58; N, 9.51; S, 5.44. Found: C, 66.91; H, 9.44; N, 9.42; S, 5.11.

Results and Discussion

UV–Vis Spectroscopy. The position of the long-wavelength absorption band of azo dyes is sensitive to medium effects; therefore, they can be used as solvatochromic micropolarity reporter molecules. For example, the wavelength of maximum absorption of MO is positioned at 463 nm in water, whereas it is situated at 417 nm in ethanol.^{2e} Similarly, upon binding of azo dyes to hydrophobic aggregates, a shift in absorption maximum occurs to shorter wavelengths. Although several dye molecules are known to sometimes spontaneously aggregate in aqueous solution, the dyes used in the present study obey Beer's law in the employed concentration ranges. Although the absorption of MO shows a break at 0.5 mM, the absorption spectrum of MO fails to show a distinct dimer band upon increasing dye concentration.^{16,31} Increasing the concentration of MO does, however, result in a blue shift of the long wavelength absorption band.¹⁶ On the other hand, dyes like thionine³² and methylene blue³³ are known to aggregate in aqueous solution, resulting in the appearance of a blue-shifted aggregate band.

Absorption spectra of the azo dyes have been recorded at different concentrations of surfactant. The spectra of MO upon addition of different concentrations of C_{12} TAB are shown in Figure 1. Successive additions of small concentrations of surfactant decrease the absorption of the band at 463 nm. Further addition of surfactant gives rise to a new band at ca. 380 nm. The absorption spectrum shows the new band as a shoulder at a concentration of 4 mM of C_{12} TAB, which is more than 3 times lower than its cmc (13.3 mM).³⁴ The intensity of the new band first increases upon further increasing the surfactant concentration and then decreases until a band at ca. 430 nm appears, characteristic for MO bound to cationic micelles.³⁵

The wavelengths of maximum absorption of MO upon addition of C_{18} TAB, C_{16} TAB, C_{14} TAB, C_{12} TAB, and C_{10} -TAB in aqueous solution at a MO concentration of 25 μM

are presented in Figure 2a. The effect of C_{18} TAB, C_{16} TAB, and C_{14} TAB is similar to that of C_{12} TAB; at concentrations considerably below the cmc of the surfactants, a strong interaction occurs that is reflected by the appearance of the short-wavelength absorption band. Again, upon increasing surfactant concentration this short-wavelength absorption band gradually disappears at the expense of the micellar band. The interactions are absent for C_{10} -TAB. However, upon increasing the dye concentration, the short-wavelength absorption band indeed appears in the absorption spectrum of MO. Table 1 compares the cmc of C_n TAB with the aggregation concentration of C_n -TAB and MO at a MO concentration of 25 μM .

Similar results were obtained from experiments using 4-*n*-alkyl-1-methylpyridinium iodide surfactants as shown in Figure 2b. In aqueous solutions of low concentrations of C_{14} pyI, C_{12} pyI, and C_{10} pyI, aggregation occurs with MO, whereas interactions are absent at low concentrations of C_8 pyI. The length of the total apolar moiety of C_8 pyI is comparable to that of C_{10} TAB, and in both cases, aggregation at low surfactant concentration is absent (at a MO concentration of 25 μM). The dependence of the aggregation process on the alkyl chain length of the surfactant indicates that hydrophobic interactions are important in determining the intermolecular interactions.

The short-wavelength absorption band also appears in absorption spectra of solutions of MO and dodecylamine hydrochloride (DAHCl), the latter differing from C_{12} TAB in its headgroup being ammonium instead of trimethylammonium. The short-wavelength absorption band appears already at a DAHCl concentration of 0.4 mM, whereas in solutions of C_{12} TAB, it appeared at 4 mM. The lowering of the aggregation concentration in the case of DAHCl can be attributed to an increase in electrostatic interactions relative to C_{12} TAB but might, in part, also be explained by a contribution of hydrogen-bonding interactions between the ammonium headgroup and the sulfonate group in the dye molecule.

Parts c and d of Figure 2 show similar results for the addition of *n*-alkyltrimethylammonium bromide amphiphiles to pMR and EO, respectively, in aqueous solution at pH 9.4. Note that low concentrations of C_{10} TAB induce a short-wavelength absorption band in the spectrum of EO, whereas it is absent in the case of MO and pMR (at similar dye concentration). In the case of C_{10} TAB, the short-wavelength absorption band in the spectrum of EO is positioned at longer wavelengths than those observed with amphiphiles possessing a longer hydrocarbon tail. Again, increasing the EO concentration decreases the short-wavelength absorption band until 395 nm, similar to that observed for the other amphiphiles studied. These results are again fully reconcilable with the role of hydrophobic interactions in the aggregation process. This is confirmed by the fact that the interactions between MO and C_{12} TAB are absent in ethanolic solutions. Solvophobic interactions between solute molecules are decreased in nonaqueous solvents such as dimethylformamide, toluene, and ethanol,³⁶ although recently vesicle formation has been reported for ethanol–water solutions and for pure ethanol.³⁷ On the other hand, electrostatic interactions in ethanol are stronger than those in water on the basis of the dielectric constants of both solvents (24.3 for ethanol and 78.5 for water),³⁸ but apparently no aggregation results from this effect. Moreover, the wavelength of

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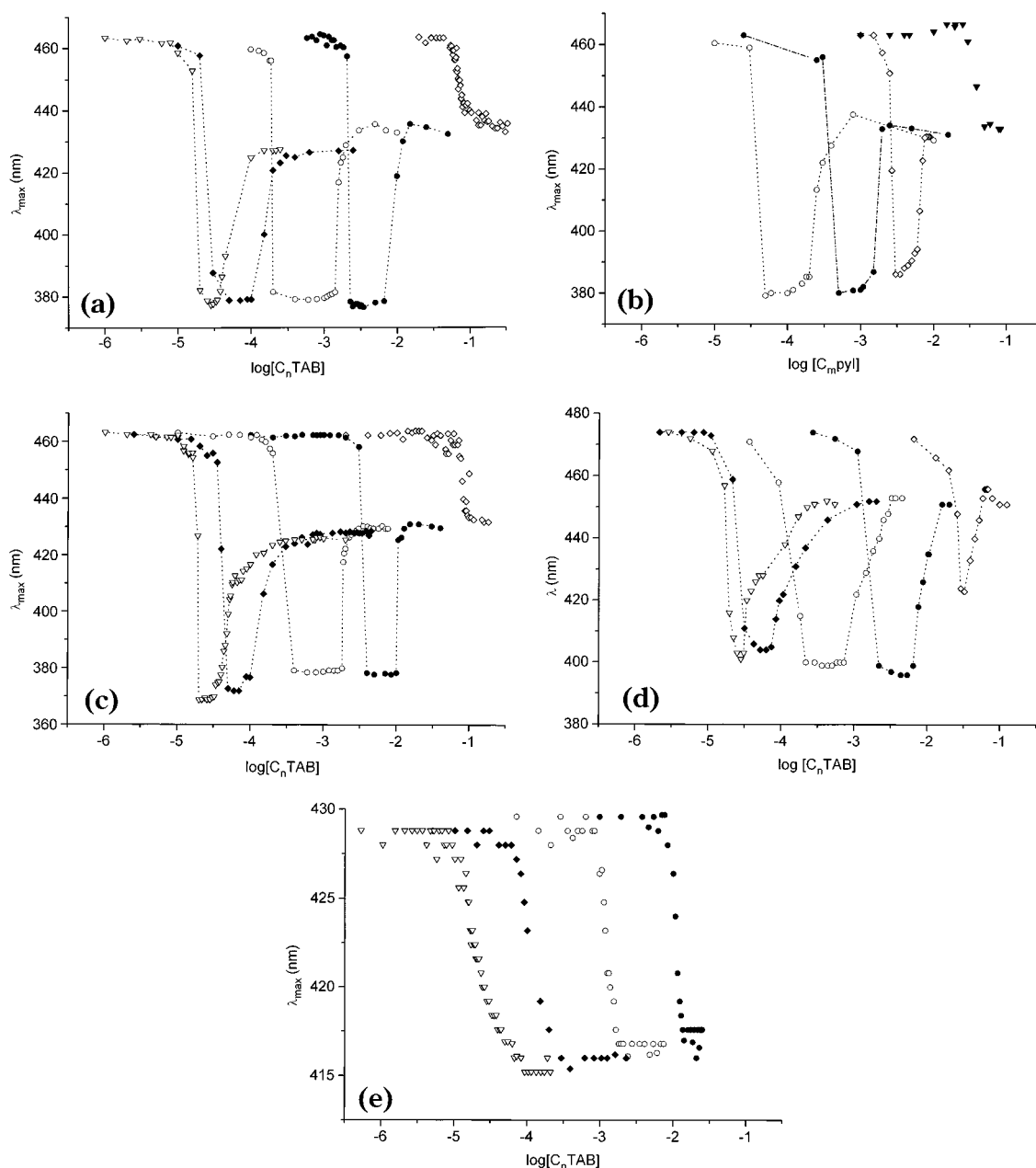


Figure 2. Effect of cationic amphiphiles on the position of the wavelength of maximum absorption of azo dyes in aqueous solution at 30 °C, pH 9.4, [MO] = [pMR] = [MR] = 25 μ M, [EO] = 23 μ M: (a) effect of C_n TAB on the absorption spectrum of MO, (\diamond) n = 10, (\bullet) n = 12, (\circ) n = 14, (\blacklozenge) n = 16, (∇) n = 18, the symbols in parts c, d, and e have the same meaning as those in part a; (b) effect of C_m pyI on the absorption spectrum of MO, (\blacktriangledown) m = 8, (\diamond) n = 10, (\bullet) n = 12, (\circ) n = 14; (c) effect of C_n TAB on the absorption spectrum of pMR; (d) EO; (e) MR. Measurements on solutions containing C_{18} TAB were performed at 35 °C.

Table 1. Aggregation Concentration of C_n TAB with MO in 25 μ M MO Solution and Critical Micelle Concentrations of C_n TAB

C_n TAB	cac (C_n TA–MO) (mM)	cmc (C_n TAB) (mM)
n = 12	2.09	13.3
n = 14	0.159	4.41
n = 16	0.024	1.0
n = 18	0.014	2.92

maximum absorption of MO in water only slightly increases upon addition of tetramethylammonium bromide (TMAB). A red shift of 4 nm was observed upon addition of 3.1 M TMAB. This again confirms that hydrophobic interactions are important for aggregation.

To examine the importance of the presence and position of the ionic group in the dye molecule, the effects of surfactants on the absorption spectrum of MR and MY

were studied. In the MR molecule, the carboxylate group is positioned ortho with respect to the azo bridge, whereas in MY an ionic group is lacking. Although the MR molecule is still fully conjugated, its wavelength of maximum absorption in aqueous solution is smaller than that for pMR. Presumably, forcing the aromatic system out of planarity results in a hypsochromic shift of the absorption band.³⁹ Both dyes do not undergo spectral shifts at low surfactant concentrations. The position of the absorption maximum gradually shifts from that in water to that in micellar solution. Figure 2e presents results of experiments using MR. Geometric reasons probably prevent association in this case. It might be suggested that in the case of MY, the electrostatic component in the aggregation process is missing, thereby preventing efficient associa-

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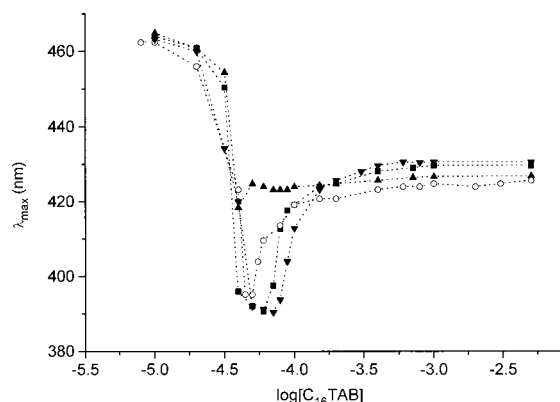


Figure 3. Effect of ionic strength on the position of the wavelength of maximum absorption of MO in aqueous solution (pH 9.4) at 30 °C of C₁₆TAB: (▼) 0.01 M NaCl, (■) 0.1 M NaCl, (○) 0.3 M NaCl, (▲) 0.75 M NaCl.

Table 2. Wavelengths of Maximum Absorption of the Dyes in Different Media

dye	water λ_{\max} (nm)	micelles λ_{\max} (nm)	low wavelength absorption band (nm)
MO	463	431	ca. 380
EO	472	452	ca. 395
pMR	463	428	ca. 375
MR	429	416	<i>a</i>
MY	440	420	<i>a</i>
ABS	318	325	<i>a</i>

^a Not observed.

tion. However, in the case of ABS (with a sulfonate group but without the dimethylamino substituent), the aggregation at low surfactant concentration was also absent. This indicates that both the dialkylamino substituent and an ionic group are prerequisites for efficient binding. In a study of the interactions of bovine and human serum albumin with several azobenzene anions, it has been observed that the isomeric position rather than the nature of the anionic substituent is important.^{2d}

Effect of Ionic Strength on Aggregation. The aggregation process is strongly influenced by the ionic strength. Figure 3 shows the effect of C₁₆TAB on the position of the absorption maximum of MO in the presence of different concentrations of sodium chloride. The short-wavelength absorption band observed at low concentrations of C₁₆TAB is diminished upon increasing the ionic strength, probably through an increase in halide counterion binding. The sensitivity to NaCl reflects the importance of electrostatic interactions in the aggregation process.^{40,41} In addition, interactions between anionic surfactants (e.g., SDS) and MO are absent,¹¹ whereas cationic surfactants with comparable alkyl chain length (e.g., C₁₂TAB) do induce changes in the absorption spectrum of MO.

Table 2 presents the wavelengths of maximum absorption of the dyes in aqueous solutions of the used surfactants.

Precipitation. In aqueous solutions of MO and pMR and surfactants (at concentrations below the cmc), precipitation occurs.⁴² Using ¹H NMR it was shown that crystals formed from aqueous solutions of C_nTAB and MO and pMR and from solutions of C_mpyI and MO consist of

Table 3. Temperature of Myelin Formation (T_{myelin}) and Melting Points (mp) of *n*-Alkyltrimethylammonium–MO Salts

salt	T_{myelin} (°C) ^a	mp (°C)
C ₁₀ TA–MO	43	237–239
C ₁₂ TA–MO	62	236–239
C ₁₄ TA–MO	68	230–232
C ₁₆ TA–MO	72	227–229
C ₁₈ TA–MO	74	220–222

^a Myelins are stable up to 100 °C.

an equimolar ratio of surfactant and dye. Elemental analysis (see Experimental Section) performed on the material that precipitated from an aqueous solution of C₁₆TAB and MO confirmed this result. In contrast to MO and pMR, crystallization does not occur in aqueous solutions of EO at low concentrations of surfactant and solutions are stable for more than 1 month. Probably ordering into dye–surfactant crystals is less efficient for EO with its *diethylamino* group compared to MO and pMR with a less bulky *dimethylamino* group. However, upon increasing the concentration of EO (above ca. 0.7 mM), precipitation does occur. Analysis of crystals precipitated from a solution of C₁₆TAB and EO revealed a 1:1 molar ratio of surfactant and dye. Crystallization has been observed before in aqueous solutions of cationic amphiphiles and methyl orange.^{16,43} Precipitation of dye–surfactant salts in a 1:1 molar ratio has been observed before in aqueous solutions of cationic surfactants and anionic dyes as well as in solutions of anionic surfactants and cationic dyes.^{17b}

Aggregation Behavior of C_nTA–MO. The aggregation behavior of C_nTA–MO in water was studied using optical microscopy and electron microscopy. The critical aggregation concentration was determined by conductivity experiments. Under a polarizing microscope, the change from solid material to closed bilayer structures in water can be followed. The precipitate was isolated from aqueous solutions of MO and cationic surfactants. Before precipitation occurred, the absorption spectrum of the solutions showed the short-wavelength absorption band. The alkyltrimethylammonium–MO crystals were subjected to a so-called phase penetration experiment.⁴⁴ Water was brought into contact with the crystals, and upon heating of the sample, the formation of myelin structures emerging at the boundary with water was examined using a polarizing microscope. Table 3 presents temperatures at which myelins appeared (T_{myelin}) and melting points. As expected, T_{myelin} increases upon increasing the alkyl chain length of the surfactant in the surfactant–MO salt. Note that the myelins are stable up to 100 °C. Myelins have been proposed to consist of wormlike vesicles in which the bilayers alternating with water layers are concentrically arranged around a central core axis of water.⁴⁵ Melting points (Table 3) decrease upon increasing the alkyl chain length of the amphiphiles; that is, increasing the alkyl chain length decreases the packing efficiency and consequently the stability of the surfactant–MO salts in the solid.

The formation of myelin structures is common to many compounds forming lamellar phases. Therefore, the morphology of aggregates formed from C₁₀TA–MO and C₁₂TA–MO crystals in water was studied by electron microscopy. The material was dissolved in water at 50 °C

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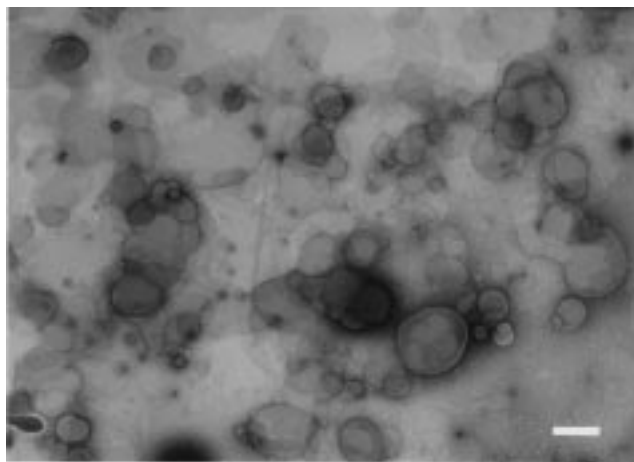


Figure 4. Negatively stained electron micrograph of vesicles formed from C₁₀TA–MO. The bar represents 1 μm.

in the case of C₁₀TA–MO and at 70 °C in the case of C₁₂TA–MO under stirring. Figure 4 shows an electron micrograph of a 5.2 mM aqueous solution of C₁₀TA–MO. Vesicles with a diameter of 400–1000 nm are observed. Vesicles formed from C₁₂TA–MO are of similar size. Vesicle solutions are stable for more than 1 week when kept above T_{myelin} but readily flocculate upon cooling.

It is tempting to view surfactant–MO salts as catanionic surfactants. Characteristic for catanionic surfactants is the formation of vesicles in mixtures of the two, whereas micelles are formed in aqueous solutions of the separate surfactants.⁴⁶ However, MO is not a surfactant molecule since it lacks surfactant properties such as effective lowering of the surface tension and a well-defined critical aggregation concentration.⁴⁷ Nevertheless, it forms vesicles in combination with an oppositely charged surfactant analogous to catanionic surfactants. Moreover, vesicles prepared in the present study form without the input of significant mechanical agitation, a property characteristic of catanionic vesicles.⁴⁶ We contend that the dye molecule should rather be viewed as a hydrophobic counterion. As a result, the packing parameter of the surfactant molecule (P), which usually determines the type of aggregate formed by a surfactant molecule,⁴⁸ increases by increasing the volume of the hydrocarbon part of the surfactant (V). Furthermore, the mean cross-sectional headgroup surface area (a_0) decreases considerably⁴⁹ (eq 1; l is the alkyl chain length). As a result of the increase in P , the novel surfactant displays vesicle instead of micelle formation. A value of $P < 1/3$ is indicative of the formation of micelles, $1/3 < P < 1/2$ indicates the formation of wormlike micelles. Vesicles are usually formed at $1/2 < P < 1$. Counterions can have

$$P = \frac{V}{a_0 l} \quad (1)$$

a large influence on the aggregation behavior of surfactants. Gamboa and Sepúlveda⁵⁰ studied the effect of counterions on the aggregation behavior of cetyltri-

methyammonium surfactants. They observed a transition from spherical to wormlike micelles upon addition of tosylate, nitrate, and benzene sulfonate anions to cetyltrimethylammonium bromide surfactants, indicating a packing parameter of $1/3 < P < 1/2$ for the surfactant monomer with strongly bound counterions. In the case of cetyltrimethylammonium hydroxynaphthalenecarboxylate, vesicles are formed in aqueous solution,⁵¹ which indicates that the surfactant monomer has a cylindrical shape. The hydrophobic MO molecule is expected to have a high degree of counterion binding leading to a cylindrical shape ($1/2 < P < 1$) of the surfactant monomer (C_{*n*}TA–MO). Apparently, the shape of alkyltrimethylammonium surfactants and thus the morphology of the aggregate formed can be tuned by choosing suitable counterions.

The critical vesicle concentration of C₁₀TA–MO as determined by conductometry is 0.84 mM at 50 °C. The aggregation concentration is dramatically lowered compared to that of pure C₁₀TAB, which forms micelles at a concentration of 60.2 mM.³⁴ Synergism in surface-active properties is a general feature of mixtures of cationic and anionic surfactants.⁴⁶ The degree of counterion binding (β) as calculated from the slopes before and after the cvc in the plot of conductivity versus surfactant concentration is 91%. This high degree of counterion binding implies the formation of aggregates in which both components are present in (almost) equal quantities. As observed by UV–vis spectroscopy at 50 °C, the short-wavelength absorption band in C₁₀TA–MO solutions appears as a shoulder in the absorption spectrum of MO at the cvc of C₁₀TA–MO. Increasing the C₁₀TA–MO concentration increases the intensity of the short-wavelength absorption band. This implies that the short-wavelength absorption band in the spectrum of aqueous solutions of C₁₀TA–MO is caused by the formation of vesicles. It seems, however, unlikely that the short-wavelength absorption band in the UV spectra of low concentrations of MO, pMR, and EO upon addition of small amounts of cationic surfactant is also caused by the formation of vesicles, which precipitate since measurements are performed below the Krafft temperature⁵² of the surfactant–dye salts. In these experiments, the concentration of dye is too low to induce the formation of vesicles consisting of surfactant and dye. The absorption spectrum of 50 μM MO shows the short-wavelength absorption band at 3 mM C₁₀TAB, whereas the cvc of C₁₀TA–MO is 0.84 mM. However, it is likely that the molecular origin of the short-wavelength absorption band is the same in both cases.

The exact origin of the short-wavelength absorption band is still not clear. It seems unlikely to explain the shift in terms of a polarity effect, since the shape of the new absorption band differs from that observed upon changing solvent polarity. A conformational change of the chromophore is also unlikely; resonance Raman spectroscopy on aqueous solutions of C₁₆TAB and MO showing the short-wavelength absorption band revealed a band around 1500 cm^{−1} characteristic for the trans form of the MO molecule.¹⁹ Also, the formation of surfactant–dye ion pairs as proposed by Dutta and Bhat²⁰ seems implausible because there is no reason the ion pair would only form at low concentrations of surfactant. It seems more likely that the hypsochromic shift observed in the spectrum of

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MO, EO, and pMR upon addition of small amounts of cationic surfactant is caused by the formation of dye aggregates^{16,53} that are bound to surfactant aggregates. The absence of isosbestic points in overlay spectra (e.g., Figure 1) suggests the formation of higher-order dye aggregates. Although the formation of several dye aggregates (except for MO) is well-known and is often reflected by an absorption band blue-shifted relative to the absorption band of the monomeric dye molecule,^{32,33} the absorption spectrum of MO does not show a dimer band upon increasing dye concentration. However, the asymmetric long-wavelength absorption band of MO in pure water might hide an aggregate band indicating that dye aggregates are already present at low MO concentrations.¹⁶ The formation of dye aggregates induced by surfactants is supported by the fact that the binding of MO to cationic polymers in water (here also the short-wavelength absorption band is observed) is cooperative.⁵ An MO molecule that binds to the cationic polymer creates a more hydrophobic binding site and facilitates the binding of another dye molecule. This implies that next to electrostatic interactions hydrophobic stacking of the aromatic parts of the azo dyes is also important in the aggregation process. The change in wavelength of maximum absorption in the spectrum of MO from ca. 380 nm to ca. 430 nm can be explained by dilution of the dye aggregates over surfactant micelles, and consequently, the spectrum of monomeric MO in a hydrophobic medium is observed. It appears likely that the dye aggregates are H-aggregates in which the chromophores are aggregated in a parallel fashion.⁵⁴ Characteristic for H-aggregates is the blue shift of the absorption band of the chromophoric unit due to favorable $\pi-\pi$ stacking interactions of the chromophores. Moreover, the new absorption band is narrower and has lower intensity than that of pure monomeric MO, analogous to literature data.⁵⁵ Similar results have been obtained in studies on amphiphiles carrying an azobenzene chromophore unit.^{55,56} Here, aggregation is reflected by a hypsochromic shift of the absorption maximum of the chromophore. Although the

formation of H-aggregates provides an explanation for the blue shift observed in the spectrum of MO upon addition of small amounts of cationic surfactant, further studies are necessary for a more detailed understanding of the nature of the blue-shifted absorption band.

Conclusions

In the present study, the interactions of several *n*-alkyltrimethylammonium bromide and 4-*n*-alkyl-1-methylpyridinium iodide amphiphiles with a number of azo dyes have been investigated. Aggregation of C₁₂TAB, C₁₄TAB, C₁₆TAB, C₁₈TAB, C₁₀pyI, C₁₂pyI, and C₁₄pyI with MO, EO, and pMR occurs at concentrations far below the cmc and is reflected by a ca. 80 nm blue shift of the $\pi-\pi^*$ absorption band of the chromophores. In the case of C₁₀TAB and C₈pyI, higher dye concentrations are necessary to induce aggregation. Interactions of MR, MY, and ABS with cationic surfactants are absent below the surfactants' cmc.

Upon increasing surfactant concentration, solubilization of the aggregates occurs into surfactant micelles. The importance of hydrophobic interactions is revealed by the chain length dependence of the aggregation process and by the observation that binding is absent in ethanol. The importance of electrostatic interactions is evident from the influence of NaCl on the aggregation process.

Precipitates isolated from aqueous solutions of C_{*n*}TAB (*n* = 10, 12, 14, 16, 18) and MO and pMR and from solutions of C_{*m*}pyI (*m* = 10, 12, 14) and MO have been shown to consist of an equimolar ratio of surfactant and dye. Using optical microscopy, the formation of myelin structures from C_{*n*}TA-MO salts has been demonstrated. Myelin temperatures have been determined by optical microscopy. Krafft temperatures increase upon increasing the alkyl chain length of the surfactant. Melting points decrease upon increasing the alkyl chain length of the amphiphiles. The formation of vesicles from C₁₀TA-MO crystals and from C₁₂TA-MO crystals has been demonstrated using electron microscopy. Vesicles are stable above their Krafft temperature but precipitate upon cooling.

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